Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine¹–³

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ABSTRACT

Background: Basal metabolic rate (BMR) is the largest component of daily energy demand in Western societies. Previous studies indicated that BMR is highly variable, but the cause of this variation is disputed. All studies agree that variation in fat-free mass (FFM) plays a major role, but effects of fat mass (FM), age, sex, and the hormones leptin, triiodothyronine (T₃), and thyroxine (T₄) remain uncertain.

Objective: We partitioned the variance in BMR into within- and between-subject effects and explored the roles of FFM, FM, bone mineral content, sex, age, and circulating concentrations of plasma leptin, T₃, and T₄.

Design: This was a cross-sectional study of 150 white adults from northeast Scotland, United Kingdom.

Results: Only 2% of the observed variability in BMR was attributable to within-subject effects, of which 0.5% was analytic error. Of the remaining variance, which reflected between-subject effects, 63% was explained by FFM, 6% by FM, and 2% by age. The effects of sex and bone mineral content were not significant (P > 0.05). Twenty-six percent of the variance remained unexplained. This variation was not associated with concentrations of circulating leptin or T₃. T₄ was not significant in women but explained 25% of the residual variance in men.

Conclusions: Our data confirm that both FFM and FM are significant contributors to BMR. When the effect of FM on BMR is removed, any association with leptin concentrations disappears, which suggests that previous links between circulating leptin concentrations and BMR occurred only because of inadequate control for the effects of FM. Am J Clin Nutr 2005;82:941–8.

KEY WORDS Obesity, metabolism, hormones, health, body composition

INTRODUCTION

The major factor determining basal metabolic rate (BMR) is fat-free mass (FFM) (1–3), with some studies finding an additional contribution of fat mass (FM) (4, 5), but others failing to find such an effect (6, 7). Even when these factors are known, however, substantial residual variation remains. Understanding the physiologic nature of this variability is important because it has been implicated in the obesity epidemic in 2 separate ways. First, low BMR (after mass effects are removed) may be a predisposing risk factor for the development of obesity (8–10), and cross-sectional studies often report that formerly obese subjects have BMRs that are 3–5% lower than expected (11). However, although some long-term studies have indicated that variation in BMR is associated with subsequent weight gain (12), other longitudinal studies have failed to replicate this effect (13, 14). Second, BMR may show an adaptive response to caloric restriction, which may predispose individuals to subsequent weight regain. Many studies have called into question whether such changes reflect metabolic adaptation or simply disproportional losses of metabolically active FFM (15–18).

Whatever the role of variations in BMR in the etiology of obesity, or the responses to caloric restriction, it is the largest component of the daily energy budget in westernized societies (19, 20). As such, manipulating BMR may provide a route toward treatment or prevention of obesity. A primary conclusion of The International Dietary Energy Consultancy Group workshop on energy and protein requirements in 1994 (20) was that “There is a need to investigate the causes of the relatively large inter-individual variations in BMR.” Despite this need being identified a decade ago, however, little progress has been made.

Leptin is an adipokine produced predominantly by adipose tissue (21). The role of leptin as a signaling molecule indicating the level of body fatness or historical levels of energy balance is well established (22, 23). Despite the broad correlation between body fatness and circulating leptin concentrations, this relation shows tremendous individual variability (24, 25), which may drive individual differences in residual BMR. Previous studies addressing the relation between circulating leptin and BMR have, however, produced confused results, with some studies finding positive associations (26–28), but others finding negative (29, 30) or no significant association (31–34). Some of these differences may reflect problems accounting for the confounding effects of FM on both BMR and leptin concentrations (35).
Hyper- and hypothyroid conditions lead to changes in BMR, but the effects of individual variations in thyroid status within the euthyroid range of variation remain unclear. Some studies (5, 36) have shown significant links of BMR to circulating triiodothyronine (T₃) concentrations, yet other studies have failed to find such effects (37, 38). In the present study, we aimed to define the roles of FFM, FM, age, and sex and to examine whether residual variation in BMR once these factors had been accounted for was linked to concentrations of circulating leptin and thyroid hormones [T₃ and thyroxine (T₄)].

SUBJECTS AND METHODS

Subject characteristics

One hundred fifty adults (n = 107 women, 43 men) aged between 21 and 64 y and having a body mass index (in kg/m²) in the range of 16.7–49.3 were recruited (Table 1) through newspaper advertisements to participate in a study investigating genetic and environmental influences on body weight. The subject group was a representative sample of the Scottish population, with 1 in 5 obese (body mass index > 30) and 54% of the population collectively overweight and obese (body mass index > 25). Demographically, Aberdeen is almost completely white, with only minor Asian communities. We did not select subjects on the basis of race, but reflecting the local demography, all the recruited subjects were white. Subjects were included only if they were not consuming any special medical diet; had a stable height and weight (weight change of no >2 kg in the previous 3 mo); were otherwise healthy on the basis of an extensive medical examination, screening blood tests (full blood count and renal, liver, and thyroid function), and electrocardiogram (ECG); and took no regularly prescribed medication or vitamin or mineral supplements. The study was approved by the Joint Ethical Committee of Grampian Health Board and The University of Aberdeen. Written informed consent was obtained.

Basal metabolic rate measurement

The subjects attended the Rowett’s Human Nutrition Unit for measurements of body composition and metabolic rate under standardized conditions. The subjects were instructed to fast overnight and to not consume caffeinated products or smoke before arriving at the unit. Subjects arrived between 0700 and 0830 and were allowed to relax for ≈30 min before measurements were made.

For estimates of basal energy requirements, BMR (MJ/d) was measured by indirect calorimetry with a ventilated hood system (Deltatrac II, MBM-200; Datex, Instrumentarium Corporation, Helsinki, Finland). The subjects were instructed not to move during the measurement period and were measured while in a supine position in a quiet room for a period of 40 min. Basal energy expenditure was calculated from the gaseous exchange rates of oxygen and carbon dioxide (39) with the use of the values during the last 20 min of the measurement period. We did not measure nitrogenous excretion but rather converted observed oxygen consumption and carbon dioxide production to energy expenditure by using the Elia & Livesey (39) equation. The error introduced by this conversion is ≈0.5% when nitrogen excretion is neglected (assuming 13% of dietary energy intake is from protein). If the subjects had unphysiologic measurements (respiratory quotient > 1.0 or < 0.73), we rescheduled them for a repeat measurement the following week and used only the repeat measure in this analysis. Repeat measurements were made on <10% of the population and were typically necessary because of hyperventilation or the subject having a cold or cough that resulted in them being restless or having abnormal breathing patterns. Calibration burns were performed on the machine at 6 monthly intervals. These indicated that the Deltatrac machine had a mean carbon dioxide recovery of 99.5% (SD = 0.5%). In a separate group of 7 subjects, we measured the within-subject variation in the BMR measurement by measuring BMR on 3 consecutive days by using the same protocol as in the main observation series. After the measurement of BMR, the subject’s height was measured to the nearest 0.1 cm with a stadiometer (Holtain Ltd, Crymych, Dyfed, Wales). Body weight was measured on a portable digital scale (DIGI DS-410; CMS Weighing Equipment, London, United Kingdom) to the nearest 50 g.

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women (n = 107)</th>
<th>Men (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>42 ± 11.4</td>
<td>47 ± 9.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.63 ± 0.06</td>
<td>1.76 ± 0.08</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.25 ± 16.5</td>
<td>84.79 ± 18.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>18.9 ± 1.0 [11]</td>
<td>— [0]</td>
</tr>
<tr>
<td>Normal weight</td>
<td>22.4 ± 1.4 [42]</td>
<td>23.2 ± 1.2 [16]</td>
</tr>
<tr>
<td>Obese</td>
<td>36.3 ± 16.5 [22]</td>
<td>38.1 ± 0.8 [7]</td>
</tr>
</tbody>
</table>

1 All values are x ± SD.
2 n in brackets.
Germany). One hundred microliters of the diluted standards, quality controls, and samples (all diluted one-third in dilution buffer) was added in duplicate to 96-well microtiter plates coated with antibody to human leptin, and 100 μL of dilution buffer was added to the blank wells. The plate was then incubated for 1 h at room temperature with shaking. An automated plate washer was used to wash the plate 3 times with wash solution, as described previously, before the addition of 100 μL of tetramethylbenzidine substrate to each well. The plate was wrapped in foil and incubated for 10 min at room temperature to allow the blue color to develop before stopping the reaction with 100 μL of 0.2 mol sulfuric acid/L. The resulting yellow color was measured at 450 nm within 5 min. The absorbance is directly proportional to the concentration of leptin. The concentration of each sample (ng/mL) was determined from a standard curve produced by plotting the absorbance values versus the leptin concentrations of the standards. The standards, quality controls, and samples were all diluted one-third, so multiplying by this dilution factor was not necessary. BioVendor provided 2 quality-control standards with expected values of 4.34 and 15.89 ng/mL. We measured each standard 5 times, yielding average values of 4.36 and 16.59 ng/mL, respectively. Thus, the average accuracy was 2.5%. Precision (CV%) across the 5 measurements was 3.5%; hence, accuracy was better than precision. All samples were run in duplicate, and the mean precision (CV %) across all samples averaged 5.2%.

Plasma triiodothyronine and thyroxine concentrations

Circulating concentrations of T3 and T4 were measured by using a rapid test kit developed by Diagnostic Automation Inc (Calabasa, CA). Fifty microliters of the standards, samples, and controls was added to a 96-well microtiter plate coated with antibody to T3 (or T4). After a 10-s mix, 100 μL of diluted (1/20) horseradish peroxidase–conjugated antibody to T3 (or T4) was added. After a further 30-s mix, the plate was incubated at 19°C to 22°C degrees for 1 h. The plate was then washed 5 times with sterile distilled water to remove unbound conjugate, and residual water droplets were removed by patting firmly onto tissue paper before the addition of 100 μL of tetramethylbenzidine substrate. The plate was gently mixed for 5 s and the blue color was allowed to develop in the dark for 20 min. The reaction was then stopped by the addition of 100 μL of stop solution and the plate was mixed for 15 s to ensure that all the blue color changed to yellow before reading the optical density at 450 nm. The intensity of the yellow color formed is directly proportional to the quantity of enzyme present and inversely proportional to the amount of unlabeled T3 (or T4) in the sample. Therefore, by reference to standards assayed under the same conditions, the concentration of T3 in the samples was quantified in ng/mL and the concentration of T4 in ng/0.5 mL. No quality-control standards were available, so we could not assess the accuracy of the measurements. All samples were run in duplicate, and the mean precision (CV %) across all individuals was 2.46% for T3 and 3.6% for T4.

Statistics

The approach taken was to partition the variation into that attributable to within-subjects and between-subjects effects by using the intersubject repeatability data. We also sought to explain the observed between-subject variation by using generalized linear models with FFM and FM, height, bone mineral content, and age as covariates and sex as a factor. Subtracting the within-subject variance and the between-subject variance explained by these factors revealed an unexplained residual variation. We sought to explore how much of this residual variation could be explained by individual differences in circulating hormone concentrations, again by using a generalized linear modeling approach. All statistical analyses were performed with MINITAB version 14 (Minitab Inc, State College, PA).

RESULTS

Basal metabolic rate

The mean BMR of all subjects was 6279 kJ/d. The minimum was 4301 kJ/d and the maximum was 10 455 kJ/d. The SD was 1199 kJ/d, and the CV was 19.1%. The results of repeated measurements in a sample of 7 subjects are shown in Table 2. In this sample, the mean (±SD) BMR across individuals was 5921 ± 1459 kJ/d (n = 7), and the within-subject SD (3 repeated measurements per subject) averaged 206 kJ/d. The contribution of within-subject variance to the total variance was therefore (206²)/ (1459²) = 0.0201. About 2% of the total variance in the measurements could therefore be attributed to within-subject variation. Because the analytic precision of the Deltatrac machine judged from the recovery of carbon dioxide in the alcohol burn calibrations was 0.5%, about one-quarter of the within-subject variance could be attributed to analytic error.

Visual inspection revealed a slight positive skew in all the distributions for morphologic traits (height, weight, FFM, FM, and bone mineral content). We examined these variables for normality by using the Anderson Darling test before analysis, and all traits except height were significantly different from normal (P < 0.01). We therefore log-transformed the variables with skewed distributions before analysis. Most of the between-subject variation in BMR was explained by differences in FFM (Figure 1), which alone explained 62.3% of the variation. Once the effects of log, FM had been removed, the remaining variation was significantly related to log, FM (Figure 1; r² = 0.18). Once the combined effects of FM and FFM had been removed, there was a significant negative effect of age (y), with older
people having lower resting metabolic rates independent of any age-related changes in their body compositions, although age explained only 6.3% of the residual variation. In a multiple regression analysis, height, sex, and bone mineral content were all nonsignificant ($P > 0.05$). The best-fit least-squares regression including the 3 significant factors was as follows:

$$\log_e (BMR) \text{ kJ/d} = 0.908 + 0.619 \log_e (FFM) + 0.132 \log_e (FM) - 0.00262 \text{ age (y)} \ (I)$$

which explained 71.4% of the variation in $\log_e BMR (F = 121.34, P < 0.001)$. The partitioning of the variance in BMR in this population is illustrated in Figure 2. Most of the variation resided between subjects, with relatively little variance due to within-subject and analytic error effects. Of the between-subject variance, most was explained by FFM, with relatively minor contributions of FM and age. There was a large unexplained residual variation between individuals that accounted for 26% of the total variance.

### Circulating leptin, triiodothyronine, and thyroxine concentrations

We obtained assay results for circulating leptin for 145 of the 150 subjects. Concentrations of circulating leptin (ng/mL) were positively related to FM ($F = 121.34, P < 0.001$) and sex ($P < 0.03$), and a large portion of the variance remained unexplained. Effects of potential predictor variables were evaluated by using generalized linear modeling.

### Relations between residual BMR and hormone concentrations

We removed the effects of FFM, FM, and age on BMR to derive a residual variance. We then sought associations between this residual variation and in circulating leptin concentrations (after removing the effects of FM and sex) and concentrations of $T_3$ and $T_4$. There was no significant association between residual variation in BMR and residual variation in circulating leptin concentrations (Figure 4; $P = 0.455$) and no relation to circulating concentrations of $T_3$ (Figure 5; $P = 0.532$). For $T_4$ concentrations, however, the relation to residual BMR was more complex (Figure 5; $P = 0.199$). Using a general linear model, we found a significant overall effect of $T_4$ concentrations ($F = 8.07, P < 0.005$) but also an effect of sex ($F = 5.68, P = 0.019$) and an interaction between these effects.
At any given T₄ concentration, men on average had higher residual BMRs. The relation between circulating T₄ and residual BMR was highly significant in the men ($r^2 = 0.25, F = 13.02, P = 0.001$) but was not significant in the women ($r^2 = 0.0, F = 0.01, P = 0.904$). Removing the single point in Figure 5 that had a large influence on the regression for men diminished but did not abolish the significance of the relation.

**DISCUSSION**

Many previous studies have quantified the repeatability of BMR measurements when using hood-based measurement systems. Previous estimates of within-subject repeatability fell in the range of 2–4% of the mean estimate (reviewed in reference 20), with the extent of repeatability depending on the interval between measurements. Our estimate of repeatability of 2.8% falls within the expected range given the short time scale between repeated measurements in our study. Because BMR is a highly repeatable trait, the contribution of within-subject variation to the total variance was trivially small (2%), which indicates that most of the variance in BMR resided between the individuals under study.

Consistent with previous studies (1–3, 42) the dominant factor influencing this variation between individuals was the extent to
which the individuals varied in their FFM. The additional role played by FM as an independent factor influencing BMR has been a matter of previous debate, with some studies finding a significant independent effect (4, 5, 43) and others failing to replicate this result (6, 7). Our studies confirmed that, in this sample of adults, FM was an independent factor—although the contribution to the total variance that was explained by differences in FM was relatively small compared with that explained by FFM. In the derived multiple regression, the coefficient relating BMR to FFM was 0.619, compared with 0.132 for the coefficient relative to FM. This suggests that each kilogram of lean tissue exerted about 5 times more effect on BMR than did each kilogram of fat tissue. This ratio of 5:1 is at odds with in vitro estimates of the energy metabolism rates of fat and lean tissue [in both humans (44), and rodents (45)], for which the differences in energy metabolism between FM and FFM ranged from 10 to 100 fold. Nevertheless, this ratio is consistent with similar multiple regression models linking energy metabolism to body composition in rodents (46).

These data emphasize that the derived regression equation is a statistical rather than a physiologic model of the BMR. The regression coefficients do not necessarily imply that the energy expenditure of FFM is 5 times greater than the energy expenditure of FM. The coefficient with respect to FM may be greater than anticipated physiologically because the presence of FM stimulates the metabolic rate of other tissues as a result of adipokine secretions that are themselves positively associated with FM. The negative effect of age on BMR, independent of any age-related changes in body composition reported here, has been previously reported (3, 47–49).

Like all previous studies (50–55), we found enormous variation in between-subject BMR (26% of total variance) that was not explained by differences in body composition or age (or sex). This variation could not be attributed to either within-subject error or analytic error, which together only summed to 2% of the total variance (less than one-tenth of the unexplained variation). In part, this unexplained variance may reflect the inadequacies of our characterization of body composition into only 3 compartments (FFM, FM, and bone mineral content). Clearly, FFM is not a homogeneous tissue (55), and, consequently, variations in the size of the organs contributing to FFM might result in variations in BMR that our analysis would not detect. However, studies using magnetic resonance imaging to model the contributions of different organs that make up the FFM (56) also leave a relatively large residual variation unexplained, although the mean predicted and observed values across a sample of individuals are close. To further explore the potential effects of unquantified variability in organ tissue sizes to the unexplained variation, we modeled the effect of variation in brain size on the BMR. In making this calculation, we assume that the dual-energy X-ray absorptiometry machine is blind to any variation in brain size and thus that brain size variations can contribute to variation in BMR, but not FFM or FM. Elia (57) suggested that the metabolism of the brain averages 1008 kJ · kg⁻¹ · d⁻¹. Gallagher et al (56) estimated the variation (SD) in the brain size of adults, measured by magnetic resonance imaging, to be 0.14 kg in women and 0.15 kg in men. However, there was a positive correlation between brain size and body size ($r = 0.54$), so some of this variability will be linked to the variation in body size. At a fixed body size, we assumed the variation in brain size might amount to one-half of the total variation (i.e., $SD = 0.07$ kg). Multiplying this variation in brain size by the energy metabolism of brain tissue gives a contribution to variation in BMR of 70.6 kJ/d. This compares with the residual variation (SD) in BMR that amounted to 529.8 kJ/d. The percentage contribution of the undetected variance (SD²) in brain size to the residual variance in BMR (SD²) was therefore $(70.6^2/529.8^2) \times 100 = 1.3\%$. It therefore seems unlikely that undetected variation in tissue sizes of highly energetic organs was a significant contribution to the observed residual variation in BMR.

The independent effects of FM and sex on circulating leptin concentrations were consistent with previous reports (35). The large degree of individual variation about the average trend in this relation also agrees with previous observations. Studies in rodents have implicated circulating leptin as a key signal related to resting metabolism (23, 58). For example, the mutant ob/ob mouse that lacks functional leptin has a lowered resting metabolic rate and a lowered body temperature (21). Several previous studies indicated that the between-subject variation in BMR is linked to between-subject variation in leptin concentrations (26–28), but others found a negative relation (29) or no significant association (31–34). Consistent with the findings of Neuhauser-Berthold et al (35) in much older subjects, we found no association between this residual variation in circulating leptin concentrations and the residual variance in BMR once the effects of FFM, FM, and age had been removed. In other words, if an individual had a particularly high concentration of circulating leptin for their FM and sex, this had no effect on whether they had a high or low BMR for their FFM, FM, and age. This indicates that the unexplained variation between subjects in BMR could not be attributed to the variability between subjects in their leptin production by the FM. This observation in our data is consistent with the observation that humans lacking functional leptin production do not have a significantly altered BMR (59). The differences in the effects of leptin between humans and rodents may in part be because laboratory rodents are routinely kept under thermoregulatory stress at around 20 °C, which is 10 °C below the lower critical limit of the thermoneutral zone (60). Hence, the effects of leptin absence on BMR of the ob/ob mouse, and its lowered body temperature, may reflect the absence of stimulation of brown adipose tissue thermogenesis (58). In humans who have their BMR measured at thermoneutral temperatures, such effects of leptin would not be anticipated, particularly because in adults, levels of brown adipose tissue are probably not of functional significance for thermoregulation.

The effects of pathologic variations in concentrations of the thyroid hormones and metabolic rate have long been established. Hyper- and hypothyroid conditions both lead to major changes in basal metabolism. However, despite these major effects, the role of individual variations in thyroid status within the euthyroid range of variation remains unclear. Some studies (5, 36) have shown significant links of BMR to circulating $T_3$ concentrations, yet other studies have failed to find such links (37, 38). We also failed to establish any link between circulating concentrations of $T_3$ and the unexplained variation in BMR. An effect of $T_4$ concentrations on BMR in men but not women was previously described (61), although in that previous study the effect was negative: higher concentrations of $T_4$ were associated with lower BMR. The variability in previous studies with respect to the effects of $T_4$ cannot be accounted for by the different sex effects, because previous studies that showed relations included studies focused only on women (36).
between the sexes and between studies points to the complexity of the interactions on the residual variation in BMR.

The absence of significant hormone effects on residual BMR was not due to analytic precision error in the hormone measurements. To evaluate the effect of these errors on the significance of the relations between the given traits and BMR, it is necessary to consider the gradient of the estimated relation between the traits in question. When this gradient is unity, an x% precision error in the x variable will translate to an equivalent error in the y variable. Thus, if the precision error (CV) in the x variable were 30%, the maximum variation in y explained by the x variable would be \( \approx 70\% \) (100% – 30%). When the gradient declines below unity, the effect of error in the x variable becomes less and less important, until at a gradient of 0, the error in x imparts no effect on the relation. Conversely, when the gradient exceeds unity, the effect of errors in x become more significant. Hence, if the gradient were 2.0, rather than unity, the maximum variation in y explained by x would only be \( \approx 40\% \) (ie, 100% – 2 \times 30%). On the other hand, if the gradient were 0.5, the maximum expected explained variation would be \( \approx 85\% \) (100% – 0.5 \times 30%). In the case of the hormones (and body-composition traits) that we tested, the precision errors in the x variables were between 2% and 5% (see methods), and the fitted gradients on logged traits were all substantially less than unity. Thus, the amount of unexplained variation in the y trait (BMR) that could be attributed to errors in the x traits would be on the order of 1–3%. This compares with the actual unexplained variation by body composition that was 26%, and the unexplained residual variation by hormones that was \( > 95\% \) for leptin, T₄, and T₃ in women and 80% for T₄ in men. Overall, the effect of the precision errors in the assays could not account for the absence of significant relations or the high residual variation in BMR.

Like many other studies, our work has indicated a large between-individual variation in BMR that cannot be explained by morphologic characteristics, including sex and age. We show here that 2 important physiologic candidates that might explain this variability, namely, variations in circulating concentrations of leptin and T₃, do not account for this unexplained variation in a population of 150 white adults. In men, however, 25% of the residual variance was associated with variation in circulating concentrations of T₄. No equivalent effect was found in women.

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JRS, KAR, and AMJ were responsible for the study concept and design. AMJ, JSD, and SDM were responsible for the data collection and laboratory analysis. JRS was responsible for the data analysis and drafting of the manuscript. JRS, KAR, and AMJ were responsible for critical revision of the manuscript for important intellectual content. None of the authors had a conflict of interest.

REFERENCES